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Immunological Consequences of Social Stratification and Change

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During the first year of our research program, we have completed the proposed longitudinal study to evaluate psychological and physiological factors influencing immune responses in adolescent male primates. Behavioral and immune assessments were conducted on 30 male primates (adult males, castrated males, adolescent males housed alone, and adolescent males housed in pairs). Analyses focused on the effects of age, biorhythms, hormonal status, psychosocial variables, and individual attributes. In addition to the longitudinal study, we conducted a cross-sectional study on 92 monkeys to evaluate life span changes in immune responses, a study of 28 juvenile monkeys to assess the effect of prior rearing conditions on immune responses, and a pilot study on the effect of aggression on the complement system. We are now in position to conduct Year 2's studies which will focus on the immunological consequences of establishing new social relationships and the effect of disrupting familiar, stable relationships.

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ANNUAL REPORT

Office of Naval Research Contract
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March 1, 1987 - February 29, 1988

The goal of this research program is to determine the effect of psychosocial variables on immune response in adolescent male primates. The primary emphasis is on the immunological consequences of forming new social relations, the effects of disrupting stable social relations, and the influence of social factors on the physiological changes observed in response to novel environmental conditions. The studies involve longitudinal assessments of adolescent male rhesus monkeys and are designed to elucidate behavioral, hormonal, and immunological predictors of individual variation. Since there was virtually no normative data on the relationship between behavior and immune function in the rhesus monkey, Year 1 was devoted to establishing basic values essential for all subsequent studies. Several studies were conducted to determine the effects of age, biorhythms, hormonal status, social housing conditions, and individual attributes on immune responses (sources of individual variability included handedness, general health, activity patterns, prior rearing conditions, trait stability, etc.). The primary study involved a longitudinal evaluation of 30 male rhesus monkeys across the year (6 adult males, 6 castrated males, 6 adolescent males housed individually, 12 adolescent males housed in 6 pairs). Subjects were purposely maintained in a non-manipulated state in order to evaluate the replicability and long-term stability of different behavioral and immunological measures. In addition, a cross-sectional survey of 92 monkeys was conducted to establish life span changes in immune responses, a survey of 28 yearling monkeys from 4 different rearing conditions was conducted to assess the possible effect of early social experiences, and a pilot study was conducted to determine the effects of aggression on hemolytic complement activity.

AGE

Significant changes in immune responses were observed across the life span in the rhesus monkey, with the greatest changes occurring in aged males over 20 years of age (Ershler, Coe, Gravenstein, Klopp, Meyer, and Houser, in press). Marked declines in lymphocyte proliferation responses, natural killer cell activity (NK), and antibody responses to tetanus toxoid vaccination were observed in older subjects. Differences between adolescent and adult males were less overt (not apparent in lymphocyte proliferation and NK responses), but antibody responses to flu vaccination did distinguish an effect of age in males between 3-10 years. Adolescent males mounted a larger primary antibody response to trivalent flu vaccination (Connaught) than did adult males, as measured by an ELISA test that we have standardized for the rhesus monkey (Ershler, Coe, Gravenstein, and Klopp, in press). Differences in lymphocyte proliferation responses and NK activity were also noted between adolescent male monkeys and younger juveniles, with higher values observed in the more mature subjects. However, of primary importance

for the purposes of subsequent studies, we have shown that adolescent males between 3-5 years of age can be considered as immunologically equivalent subjects for use in future experiments.

BIORHYTHMS

Because of the large literature on diurnal and seasonal variation in physiological measures, we were concerned about the possible effect of endogenous biorhythms on immune responses. We decided to evaluate two timepoints in the day, early morning and late afternoon (0700-0800 and 1530-1600, respectively), since these times represented the extremes of the workday when samples might normally be obtained. All subjects were housed in a 12L:12D schedule with lights on at 0600. Blood samples were collected from the monkeys at 3 week intervals, alternating between AM and PM samples (we had originally planned on 2-week intervals, but a prior study indicated that shorter intervals between blood samples might result in an effect of repeated sampling, Ershler, Coe, Laughlin, Klopp, Gravenstein, & Roecker, in press). No significant effects of diurnal variation were detected in lymphocyte proliferation responses for any age or housing condition. There was a small, but statistically significant decline in NK activity during the late afternoon in most subjects. The possibility of a seasonal influence is currently being analyzed following completion of the 12 month series of samples in March 1988. Preliminary evaluations indicate that there isn't a marked annual rhythm in the immune responses of the rhesus monkey, which will expedite the conduct of future studies. The absence of a seasonal rhythm may be due in part to the lack of an annual hormone rhythm in these males maintained under constant laboratory conditions. Radioimmunoassay of plasma testosterone and cortisol levels did not indicate any consistent diurnal or annual changes, although there were effects of age and social dominance.

HORMONAL STATUS

When attempting to predict the physiological factors that might affect immune responses in male subjects, it was essential to consider the possible impact of gonadal hormones. For that reason, our longitudinal study included an examination of immune responses in castrated subjects. We have been unable to document any immunological differences between castrated male subjects and age-matched intact subjects, despite the existing literature on the influence of gonadal hormones on immunity. The comparison so far has included T cell markers, lymphocyte proliferation responses, NK activity, and antibody responses to flu vaccination. Analyses to correlate immune responses specifically with circulating levels of plasma testosterone and cortisol in intact subjects are currently underway. Adult males had higher levels of testosterone and lower cortisol levels than did adolescent males (5.5 vs 1.2 ng/ml, and 13.5 vs. 21.9 µg/dl, respectively). Dominant adolescent males living in pairs had significantly higher levels of testosterone and lower cortisol levels than did their subordinate social partners (1.4 vs. 0.8 ng/ml, and 21.7 vs. 24.8 µg/dl, respectively).

Given that gonadectomy did not have a significant effect on immune responses, it is unlikely that individual differences in testosterone

levels will account for a large portion of the variation in immune responses. Further, despite testosterone differences in dominant and subordinate monkeys, there were no overt differences in immune reponses in these stable pairs. It should be noted that these pairs were maintained in stable relationships over the year; we anticipate marked effects of dominance on immune responses in the newly-established pairs that will be evaluated in Year 2.

PSYCHOSOCIAL STATUS

In the original proposal, we hypothesized that psychosocial variables would have their greatest effect on immune responses during the establishment and disruption of social relationships and that the effects would subside during stable conditions. Behavioral and physiological evaluations of adolescent males maintained in stable conditions support this hypothesis. Despite clear behavioral evidence of dominance interactions in pair-housed males and a significant effect of dominance on circulating hormone levels, there were no indications of a pervasive effect of social status on immune responses. Dominance rank in the 6 stable pairs did not have a significant effect on any of the immune responses that we have evaluated. Social vs. individual housing did affect several measures, but probably not at a level that would influence disease susceptiblity. Pair-housed subjects had lower levels of T cells and a lower ratio of helper to suppressor cells (17/30%) than did individually-housed males (26/36%). In keeping with this tendency, pair-housed males tended to show lower lymphocyte proliferation responses than did individually-housed males, although the latter differences did not reach statistical significance. Antibody responses to flu vaccine did not reveal any additional differences between males in the different housing conditions. Thus, under stable conditions there does not appear to be a strong effect of social housing or dominance rank on basic immune responses in the rhesus monkey. However, based on evaluation of the immune responses during the first months of the year-long study and on the immunological data from those pairs which showed the highest levels of dominance contention, we believe that there will be immunological changes associated with alterations in social relations during Year 2's studies.

INDIVIDUAL ATTRIBUTES

An additional goal of our initial studies was to determine other predictors of individual variation in immune responses. Based on the growing evidence for a relationship betweeen brain laterality and immunity, we conducted a preliminary assessment of the correlation between handedness and immune responses. The 30 males in the longitudinal study were subjected to a series of food choice tests to establish hand preference. Males were ranked on a continuum from left- to ambidextrous to right-handed. Exceeding our expectation, there was a significant correlation between hand bias and certain immune responses, especially lymphocyte responses to Con A. Left-handed monkeys had the lowest responses, while right-handed monkeys showed higher responses than did ambidextrous monkeys. We are now in the process of developing the technology and a collaborative relationship with Dr. Richard Davidson (Department of

Psychology, UW) to record EEG and EP from scalp electrodes in order to correlate the handedness and immune data with direct measurements of brain laterality and regional activation in the monkey.

Another biological measure that has proven to have a surprisingly high correlation with immune measures is nail growth rate. We began to utilize this measure initially as a biomarker of the aging process in aged female subjects. The slowing of nail growth rate was highly correlated with the decrease in NK activity in a study of 14 aged female subjects. We have demonstrated further that nail growth rate is efficacious for monitoring aging in male monkeys across the life span. Within cohorts of similarly aged subjects, such as the adolescent males, nail growth rate proved to be positively correlated with immune responses. The faster a male's nail was growing, the higher his lymphocyte proliferation response tended to be. This easy-to-assess measure, thus, has extraordinary potential for predicting aging and basic immunity in the nonhuman primate. It should be noted that many factors can influence nail growth rate, and these observations would not have been possible if dietary factors and early rearing history were not controlled.

A final variable that will be considered in this report is the effect of early rearing conditions. We have now established that length of time that infants spend with their mothers significantly affects the responsivity of their lymphocytes to mitogens when tested at 1-2 years of age (Lubach, Coe, Ershler, and Klopp, 1987). An initial comparison of 8 mother-reared and 8 nursery-reared subjects indicated that lymphocyte proliferation responses were significantly elevated above the normal range in nursery-reared monkeys. We have now monitored these monkeys through 2.5 years of age and have found that Con A responses, in particular, continue to be skewed upward into an abnormal range. A follow-up study examined lymphocyte proliferation responses in 12 additional juveniles that had been weaned early from the mother at 6 months of age, instead of the usual time of 12 months. These subjects showed intermediate elevations between those of the normally-reared and nursery-reared juveniles. Although all of the adolescent subjects selected for the ONR research program are purposely chosen because of their normal rearing histories, we are now evaluating whether early familial or clinical histories may account for some of the individual differences in immune responses. The behavior of the 30 males included in the longitudinal study was also monitored across the year. On-going analyses are attempting to correlate individual differences in activity patterns, emotionality, self-directed vs. environment-directed behavior, sociality, etc. with the immunological data available on each subject. Similar methods will be employed in the Year 2 studies, which will track parallel changes at the behavioral and immunological levels during transitions in social relations. In addition, the immunological status of adolescent males will evaluated during environmentally-arousing and subordinating situations.

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